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The Bond between Sisters

Minireview

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The accurate segregation of chromosomes in mitosis is ensured by the association of sister chromatids from S phase until their stable orientation toward opposite poles of the metaphase spindle. Only at the onset of anaphase is the cohesion between sister chromatids released, allowing them to segregate from one another. Sister chromatid cohesion is likewise essential during meiosis, but in a more complicated manner. In meiosis, cohesion must be maintained in pericentromeric regions throughout the first (reductional) division, when homologous chromosomes segregate from one another, until anaphase of the second (equational) division, when sister chromatids segregate from one another. Moreover, if crossing over has occurred between homologous chromosomes, as it does in most meioses, cohesion must be maintained along the chromosome arms to stabilize chiasma position (Darlington, 1932; Maguire, 1974). This cohesion along the chromosome arms must be released (at least distal to chiasmata) to allow chiasma release during anaphase of the first meiotic division. Recently, the molecular analysis of a Drosophila sister chromatid cohesion gene, *mei-S332*, previously studied genetically and cytologically, has shed light on the process of meiotic sister chromatid cohesion (Kerrebrock et al., 1995 [this issue of *Cell*]).

mei-S332

The original *mei-S332* mutation was discovered by Sandler et al. (1968) in a screen of natural populations for meiotic mutations. The S in the name refers to the location at which the fly carrying this mutation was captured: a winery on the Via Saleria outside of Rome (L. Sandler and D. Lindsley had the rather inspired idea of doing their screen in Italy; see Hawley, 1993). The *mei-S332* mutation had two properties that made it unique among known Drosophila meiotic mutations. First, it caused high levels of nondisjunction that appeared to occur primarily at the second meiotic division. Second, it was the first mutant that affected the segregation of all chromosomes in both male and female meiosis and thus defined a function shared between the two sexes. This was somewhat surprising, since meiosis proceeds through fundamentally different pathways in Drosophila melanogaster males and females, and these pathways had been previously thought to be under separate genetic control. In Drosophila females, meiosis follows a typical pathway in which synapsis and exchange between homologous chromosomes allow their stable orientation toward opposite poles of the spindle. In males, however, recombination, synaptonemal complex, and chiasmata are normally absent, and chromosome pairing is based on the cohesion of specific pairing sites (McKee and Karpen, 1990).

The *mei-S332* allele was subjected to extensive genetic and cytological characterization by two of L. Sandler's students (Davis 1971; Goldstein, 1980). Goldstein (1980) showed cytologically that the defect in *mei-S332* males is manifested as premature sister chromatid separation beginning at anaphase I (Figure 1). By the time the chromosomes recondensed during prophase II, most or all pairs of sister chromatids had separated from one another. These precociously separated sister chromatids did not congress on the metaphase II plate, but rather moved at random with respect to one another. As a result, sometimes one daughter cell received both sister chromatids, leaving the other with none, or sometimes a chromatid lagged at anaphase and was excluded from both daughter nuclei. Thus, a defect in sister chromatid cohesion occurring at anaphase I was manifested genetically as apparent nondisjunction at meiosis II. Goldstein maintained that the *mei-S332* gene product was required to hold sister centromeres together from the time of kinetochore duplication at metaphase I until the onset of anaphase II. Given that holding sisters together at metaphase I must be a general property of all meiotic systems, the finding that *mei-S332* disrupted sister chromatid cohesion explained the seemingly paradoxical effect on both sexes.

In spite of the intriguing phenotype elicited by *mei-S332*, almost a decade passed before the gene was again studied, this time in the laboratory of T. Orr-Weaver. Orr-Weaver and colleagues accomplished three crucial objectives. First, they isolated several *mei-S332* alleles and deficiencies for the locus, allowing them to show that the null phenotype is similar to the phenotype of the original *mei-S332* mutation and that this gene is required to maintain sister chromatid cohesion in both sexes from anaphase of meiosis I until anaphase of meiosis II (Kerrebrock et al., 1992). Second, the locus was molecularly cloned and the primary structure of protein deduced. Third, the protein was determined to be localized to the centromeric regions of meiotic chromosomes (Kerrebrock et al., 1995).

The MEI-S332 Protein and Its Location on Meiotic Chromosomes

The *mei-S332* gene is expressed as three male-specific transcripts and one female-specific transcript, each of which is predicted to encode the same 44 kDa pioneer protein. That this protein sequence shares no significant similarities with existing sequences in the protein databases is not surprising, given the lack of known proteins with a similar function. To begin a structure–function study of MEI-S332, Kerrebrock et al. (1992) determined the molecular lesions in each mutant allele. All alleles, except for the first, had been isolated by their failure to complement the original allele in males, yet some alleles produced a much stronger phenotype in males than in females and vice versa (Kerrebrock et al., 1992). Interestingly, the mutations that cause more severe defects in male meiosis are clustered near the N-terminus, whereas the mutations that cause more severe defects in female meiosis are near the C-terminus. While the characterization of *mei-S332*

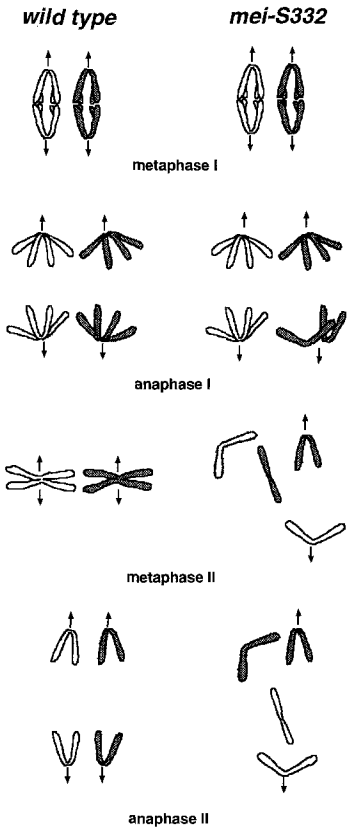

Figure 1. Chromosome Segregation during Meiosis in Wild-Type and *mei-S332* Drosophila Males

At metaphase I, sister chromatids are resolvable only in distal regions. During anaphase I, sister chromatids begin to separate precociously in *mei-S332* males (lower right). Because all sisters have disjoined by prophase II, congression on the metaphase II plate is never observed. The result is that sister chromatids are often distributed unequally to daughter cells, with lagging chromatids (anaphase II; center) being lost altogether.

indicates that at least one meiotic function, centromeric sister chromatid cohesion, is common to both male and female meiosis in Drosophila, there are apparently sex-specific differences in the utilization of this function.

One model for *mei-S332* function is that the gene product binds to centromeric regions to hold sister chromatids together. To test this model, Kerrebrock et al. (1995) determined the localization of MEI-S332 by fusing it to green fluorescent protein (GFP). In a beautiful series of fluorescent images, the authors demonstrate that the MEI-S332–GFP fusion protein stains the centromeric region of each chromosome in spermatocytes, beginning at prophase of the first meiotic division and continuing until the onset of anaphase II. Prior to chromosome condensation, the protein does not colocalize with DNA, but rather appears to be located throughout the cytoplasm. By the time the chromosomes have condensed, however, MEI-S332–GFP is found in distinct foci associated with the chromosomes. The most notable image is of anaphase I, when individual chromosome arms can be resolved and MEI-S332–GFP can be seen clearly to localize to the centromeric region near the leading edge of the migrating chromosomes. The protein remains localized to centromeric regions until the onset of anaphase II, when localized staining is suddenly lost. The striking contrast between the presence of MEI-S332–GFP on anaphase I chromosomes and its absence from anaphase II chromosomes (Figures 3C and 3F in Kerrebrock et al., 1995) provides clues to the molecular basis for the *mei-S332* mutant phenotype: MEI-S332 is an essential component of the glue that holds sister centromeres together until they segregate from one another at anaphase II.

The fusion protein apparently localizes to centromeres during female meiosis also. As pointed out by Kerrebrock et al. (1995), MEI-S332–GFP fluorescence is found in prometaphase I oocytes in up to eight distinct foci within the chromosome mass known as the karyosome. By metaphase I, the point at which meiosis arrests in Drosophila females, fluorescence is restricted to two regions at opposite ends of the karyosome. The coincidence between the number of MEI-S332–GFP foci in prometaphase and number of centromeres (eight) suggests that MEI-S332 binds specifically to centromeric regions of female meiotic chromosomes. This conclusion is strengthened by the further coincidence between the position of the metaphase staining and the positions of the metaphase centromeres (poleward edge of the karyosome). In contrast, no localization to chromosomes in Drosophila mitotic cells was observed, consistent with the absence of any somatic cell defects in flies carrying a strong allele over a deletion. Hence, MEI-S332 mediates meiosis-specific sister chromatid cohesion in the centromeric regions by specifically binding to these regions.

It is not yet known whether MEI-S332 localizes to the centromeres per se or to pericentromeric sequences. Further localization studies in flies bearing chromosome rearrangements that move, delete, or duplicate centric heterochromatin may help to resolve this point.

Centromeric Regions Required for Maintaining Sister Chromatid Cohesion during Meiosis

Are there specific centromeric sequences to which MEI-S332 binds to hold sister centromeres together? Examples of centromeric sequences required for meiotic sister chromatid cohesion have been identified in the yeasts Saccharomyces cerevisiae and Schizosaccharomyces pombe. The S. cerevisiae centromere has been divided into three elements: CDEI, an 8 bp conserved sequence; CDEII, a 78–86 bp AT-rich region; and CDEIII, a 26 bp conserved sequence (reviewed by Hegemann and Fleig, 1993; Figure 2A). A deletion of CDEI has only a small effect on mitotic centromere function, but some assays reveal precocious separation of sister centromeres in the first meiotic division or missegregation at the second division (Sears et al., 1995, and references therein). Similarly, a partial deletion of CDEII results in a high level of precocious separation of sister chromatids in meiosis I and high nondisjunction in meiosis II, yet have little effect on mitotic segregation (Sears et al., 1995). Conversely, several mutations within CDEIII that severely impair mitotic centromere function do not have strong effects on meiotic

segregation. Hence, there are meiosis-specific and mitosis-specific regions of the S. cerevisiae centromere, some of which are involved in sister chromatid cohesion in the different division types.

S. pombe centromeres are more complex, ranging in size from 40 to 100 kb, arranged as a 4–7 kb central core flanked by a large inverted repeat (Steiner et al., 1993; Figure 2A). The inverted repeat is composed of arrays of centromere-specific repeats that vary in number and relative position among different chromosomes and strains. Some segments within the inverted repeat have been demonstrated to be important in maintaining sister chromatid cohesion in the first meiotic division (Hahnenberger et al., 1991).

Are there specific *cis*-acting sequences with a similar function in Drosophila centromeres? As in other metazoans, Drosophila centromeres are embedded in megabase-length blocks of heterochromatin composed largely of simple sequence repeats, a property that has hindered molecular characterization. In a paper in a recent issue of *Cell*, Murphy and Karpen (1995) describe the molecular mapping of a Drosophila centromere carried on a minichromosome. By inducing deletions and other rearrangements on the minichromosome, the authors determined that the sequences essential for minimal centromere function span a 220 kb region, designated *Bora Bora*, that includes both simple sequence and complex DNA (Figure 2A). Completely normal transmission requires an additional 200 kb of flanking heterochromatin, on either side of the essential core. The authors suggest that this flanking region plays an important role in sister chromatid cohesion, in agreement with cytological studies in Drosophila and other organisms that also indicate such a role for centric heterochromatin (reviewed by Miyazaki and Orr-Weaver, 1994).

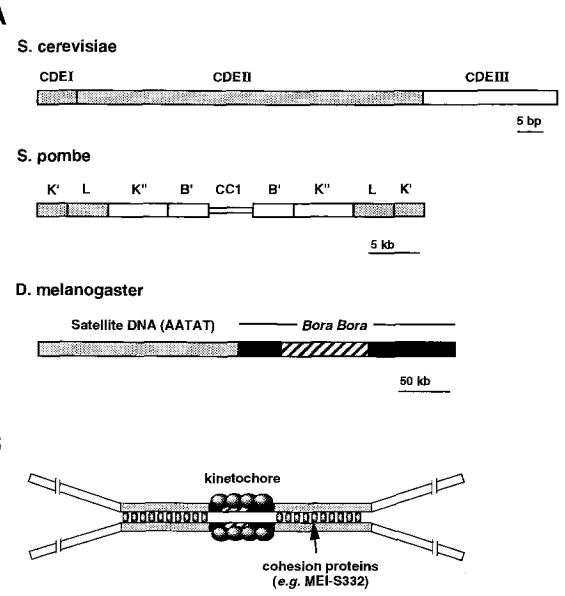

Figure 2. Centromeric Sequences Required for Sister Chromatid Cohesion

(A) Schematic of centromere structure in S. cerevisiae, S. pombe, and D. melanogaster. Regions demonstrated or believed to be required for meiotic sister chromatid cohesion are stippled. See text for descriptions of the centromeres and references. The S. pombe centromere depicted is *CEN1*. The Drosophila core centromere consists of complex DNA sequences (closed boxes) flanking a region of unknown composition (hatched box). Heterochromatin surrounding the core is thought to be involved in mediating sister chromatid cohesion, though this has not yet been demonstrated conclusively.

(B) A model for centromere function in Drosophila (Murphy and Karpen, 1995). The core centromere contains sequences to which kinetochore proteins bind. Sister chromatid cohesion proteins, such as MEI-S332, bind to heterochromatic sequences to hold the sister centromeres together until the onset of anaphase II (or mitotic anaphase). Cohesion protein-binding sites may exist on one or both sides of the core centromere and may also be interspersed within centromeric sequences.

Interestingly, Murphy and Karpen (1995) found that minichromosome derivatives that contained less than 200 kb of heterochromatin flanking the core centromere were transmitted less well through females than through males. Since this difference was shown not to depend on premeiotic mitoses, and other divisions are presumably the same in both sexes, this observation implies that there are meiosis-specific functions within the heterochromatin flanking the centromere (see also Hawley et al., 1992). One attractive model is that this flanking heterochromatin is involved in sister chromatid cohesion, perhaps by binding cohesion proteins such as MEI-S332 (Figure 2B). In this light, it will be interesting to see whether MEI-S332 binds to the deleted minichromosome derivatives in vivo or in vitro and whether MEI-S332 binds to AATAT satellite DNA, which is the principal component of the heterochromatin on at least one side of the minichromosome centromere (Le et al., 1995).

Cohesion along Meiotic Chromosome Arms

The localization of MEI-S332 by Kerrebrock et al. (1995) indicates that the protein is localized to centromeric regions by prophase I. However, genetic and cytological studies reveal no requirement for *mei-S332* until anaphase I. Thus, some other means of ensuring sister chromatid cohesion is sufficient until the onset of anaphase I. It seems likely that in the absence of MEI-S332, cohesion along the chromosome arms is sufficient to keep sister chromatids together until the onset of anaphase I. Two models have been put forward to account for sister chromatid cohesion along meiotic chromosome arms, one based on catenation produced during replication and the other on specific cohesion proteins (reviewed by Maguire, 1990; Miyazaki and Orr-Weaver, 1994; Carpenter, 1994).

In Drosophila, one candidate for a specific protein that mediates cohesion along meiotic chromosome arms is the product of the *ord* locus. Mutations in *ord*, like mutations in *mei-S332*, result in precocious separation of sister chromatids during meiosis in both sexes (Miyazaki and Orr-Weaver, 1992). However, the *ord* phenotype is manifested cytologically by prophase I (Goldstein, 1980). A likely explanation is that ORD is required to maintain cohesion along the arms until anaphase I. Prior to the onset of anaphase I, MEI-S332-mediated cohesion at the centromeres is not sufficient to overcome the absence of ORD-mediated cohesion and thereby prevent the precocious sepa-

ration of sisters. ORD-mediated cohesion is released during anaphase I, creating a requirement for MEI-S332-mediated centromeric cohesion. A characterization of the ORD protein similar to that done for MEI-S332 should yield insights into the nature of cohesion along the arms, if indeed this is where ORD acts. Taken together with further characterization of MEI-S332 and centromeric sequences, this work will undoubtedly go far toward developing a complete picture of sister chromatid cohesion and the crucial role it plays during meiosis.

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